

# Reconstruction of ancestral character states in neocoleoid cephalopods based on parsimony

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**Abstract:** The Neocoleoidea, sister group to the Belemnitoidea, includes all living cephalopod species except nautilids, as well as their immediate ancestors. Several hypotheses have been published about the morphology of ancestral neocoleoids. Ancestral states are easily inferred from fossils for some characters, such as 10 arms and the presence of an ink sac in basal coleoids or the presence of fins in ancient octopods. Many inferences are less strongly supported, though, and open to debate. We examine this problem using three cladograms resulting from analyses of morphology and DNA sequences (both mitochondrial and nuclear) from samples representing the full diversity of extant coleoids. Character states at three ancestral nodes (neocoleoid, octopodiform, and decapodiform) are reconstructed for 51 morphological characters using cladistic parsimony. Strong or moderate agreement among the three trees was found for almost 3/4 of the character-at-node reconstructions. The level of agreement among the trees varied among nodes, with strongest agreement found at the ancestral octopodiform node. However, some of these reconstructions seem unlikely to be correct. Changes in subclade resolution can exert varying effects on inferences about basal nodes. Because several subclades within the neocoleoids are not yet adequately resolved, we cannot be very confident in reconstructions of ancestral character states based solely on parsimony and we propose a provisional suite of character-state reconstructions including other sources of inference in addition to parsimony.

**Key Words:** squid, octopod, cuttlefish, morphology, evolution, phylogeny, cladistic consensus

The reconstruction of ancestral character states is an important step in unraveling the pathways of evolution and the changes that led to the present diversification in cephalopods. Naef (1921-3) relied on inference based on his extensive knowledge of comparative anatomy, embryology and paleontology to reconstruct generalized types from which all major coleoid groups could be derived. While not intending these to represent ancestral forms, he often felt that they did so. With the advent of more rigorous means of reconstruction, Naef's types are no longer acceptable for determining primitive states even though many of his conclusions ultimately may be supported. Subsequent efforts (*e. g.*, Bandel and Leich, 1986; Haas, 1997) have been based largely on inferences from fossils and the literature on anatomy of living cephalopods. Coleoid fossils, although rare, have occasionally been of use in determining the presence of some anatomical structures, such as an ink sac and ten arms in early coleoids and fins in ancient octopods (Young *et al.*, 1998). However, the fossil record of coleoids is exceptionally poor (Foote and Sepkoski, 1999), limiting its usefulness for character-state reconstruction.

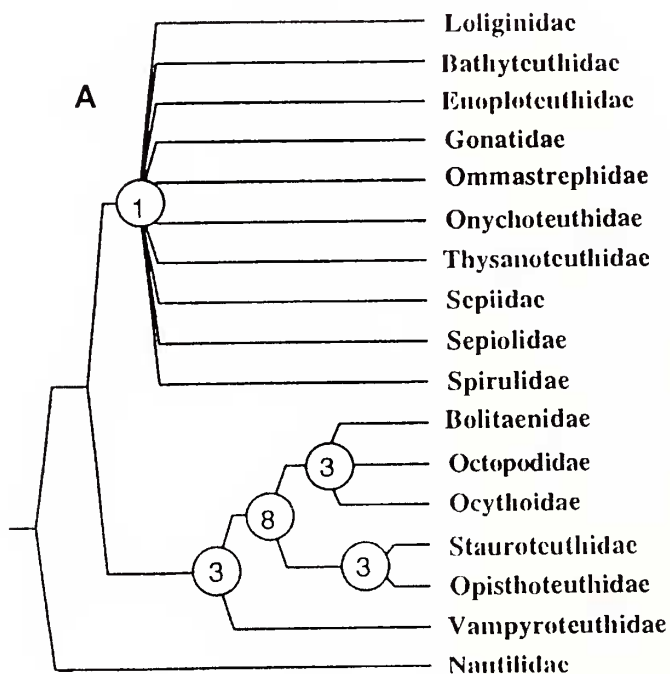
An alternative approach is to use cladistic parsimo-

ny to infer character states at ancestral nodes of a cladogram. Cladistic reconstruction is based on rules that define which character states are most likely to occur at a node (Cunningham *et al.*, 1998). However, even with carefully and accurately defined character states, the results are not definitive. Nevertheless, inferences can be robust (Shultz *et al.*, 1996). We present here the first attempt to reconstruct ancestral character states for neocoleoid cephalopods (defined by Young *et al.*, 1998 as the sister group to the belemnoids and containing all extant coleoids) using parsimony analyses of cladistic hypotheses of phylogeny based on both morphological and molecular characters. We previously analyzed 50 morphological characters in order to determine relationships among the major groups of extant coleoid cephalopods (Young and Vecchione, 1996). These characters, for the most part, are those for which we try to construct evolutionary histories, although several characters have been redefined or replaced. We use the cladistic relationships inferred from morphology by Young and Vecchione (1996) as well as molecular phylogenies based on mitochondrial (Carlini and Graves, 1999) and nuclear (Carlini, 1998) DNA sequences.

## MATERIALS AND METHODS

### Morphology

The data matrix used for the reconstructions is modified from Young and Vecchione (1996) and that paper can be consulted for much of the material examined. We have revised a few character definitions for the present analysis and have added or replaced a few characters. The character descriptions and data matrix are presented in Appendices 1-2. The consensus cladogram that Young and Vecchione considered the best estimate of neocoleoid phylogeny based on morphology is reproduced in Fig. 1. For the present study, we added Belemnitoidea, the sister group to the neocoleoids, with character states derived from literature on fossil belemnoids. As is discussed below, reconstruction by parsimony does not always infer reasonable character states at ancestral nodes. We therefore offer a set of opinions in Appendix 3 about ancestral character states. These opinions summarize the discussion of individual characters in Appendix 1. The parsimony solution is accepted unless some other source of information, such as paleontology or ontogeny, indicates that the inference from parsimony is particularly questionable.



### Molecular sequences

Taxonomic sampling and details of extraction, amplification, cloning, and sequencing methods for DNA are presented by Carlini (1998). Many alternative phylogenetic analyses, using different models for cladogenesis, substitution, weighting, and combinations of molecular data sets are presented there as well, along with cladogram parameters such as tree lengths, consistency indices, bootstrap values, etc. We have selected here what we feel are the most reasonable phylogenetic hypotheses for separate mitochondrial and nuclear DNA sequences. The cladograms for both molecular data sets are fully resolved and are the most parsimonious from unweighted parsimony analysis. For reconstruction analyses, the trees representing these hypotheses were "pruned" so that the nodal structure of the trees was retained but terminal taxa are those for which morphological character states were assessed. Although the resulting trees appear pectinate, many of the terminal branches actually represent clades on the original molecular trees. Tree statistics are presented in the captions to Figs. 2 and 3.

Mitochondrial.— The cytochrome c oxidase I (COI) cladogram (Fig. 2) is similar to that of Carlini and Graves

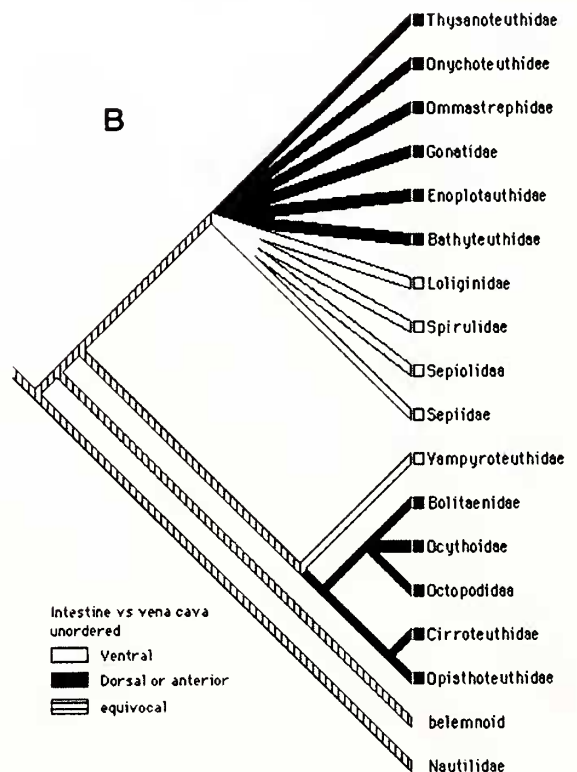


Fig. 1. **A.** Morphological consensus cladogram based on Young and Vecchione (1996); consensus of 14 trees, tree length 46, consistency index 0.98, retention index 0.99. Numbers within circles are the number of unambiguous synapomorphies supporting the node at that location. **B.** Example of reconstruction cladogram based on A. In this and the following two figures, the same character (number 47, the position of the intestine relative to the vena cava) is used as an example of character-state reconstruction.

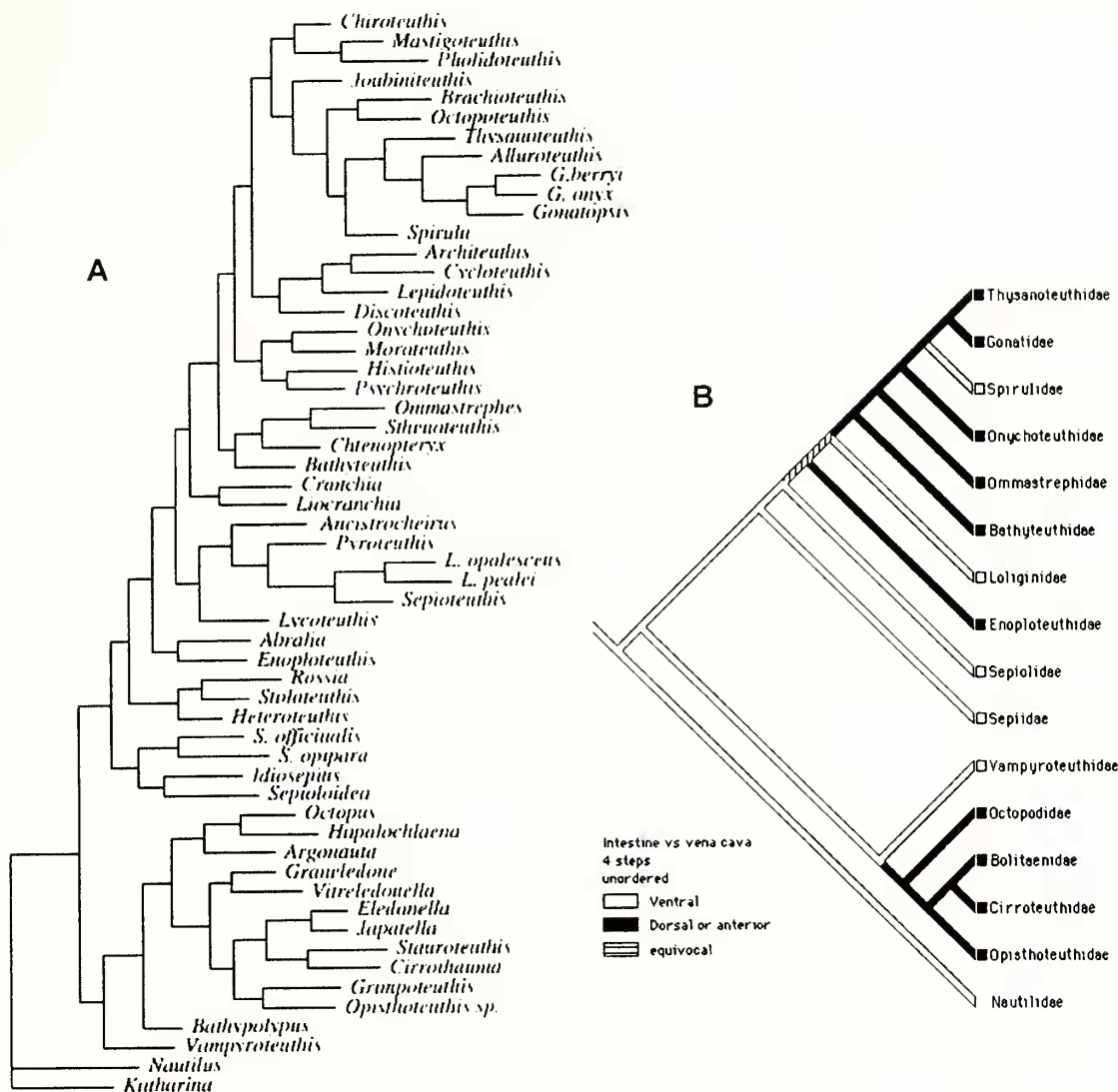


Fig. 2. A. Maximum parsimony cladogram based on DNA sequence of the mitochondrial gene for cytochrome *c* oxidase I; tree length 3763, consistency index 0.167, retention index 0.329. Branch lengths are proportional to changes in nucleotide sequence. B. Cladogram “pruned” for character-state reconstruction to retain nodal structure but reduce terminal taxa to those for which morphological data were assessed.

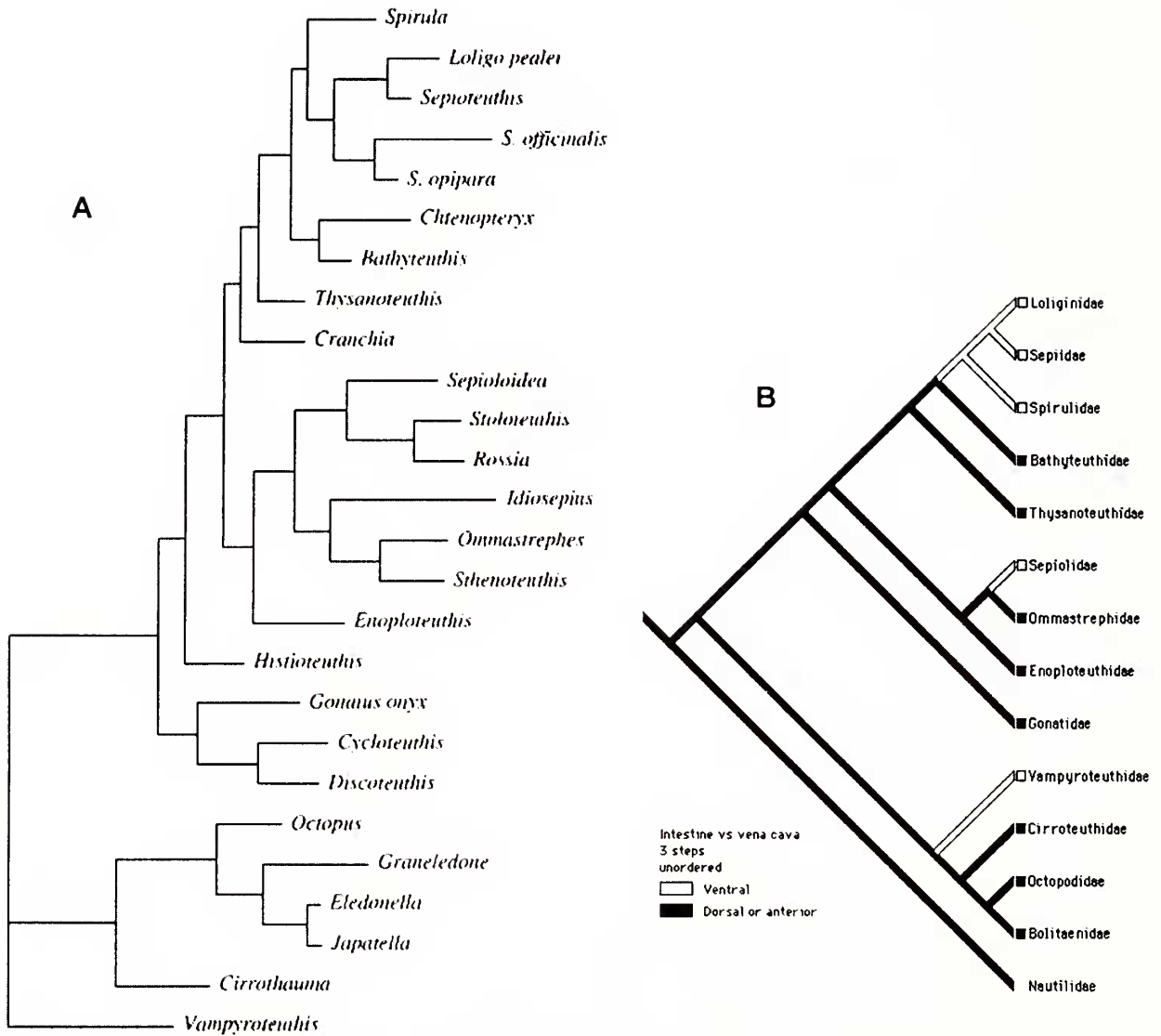
(1999), but the data were reanalyzed after including additional taxa. For the present paper, a 657 base-pair segment of the COI gene was analyzed for 55 species. Of these characters (base pairs), 297 were parsimony-informative. The result was a single most-parsimonious tree of length 3763 (Fig. 2).

**Nuclear.**—Preliminary phylogenetic analysis by Carlini (1998) on cephalopod actin sequences suggested that at least three paralogous actin genes had been amplified and cloned. These were subsequently discriminated among using restriction endonucleases. This finding was supported subsequently by genomic southern blotting, a more rigorous means of assessing gene copy number (Southern, 1975; Carlini *et al.*, 2000). Two of the three copies of the actin

gene were analyzed in detail, and gene sequences from both paralogs were obtained from 26 taxa. The two genes, designated actin I and actin II, were concatenated in each taxon for a total of 1568 base pairs in the analysis of the combined data sets. The total number of parsimony-informative characters was 376. The cladogram presented here (Fig. 3) represents the single most parsimonious tree (length = 1581) derived from cladistic analysis of the combined actin genes.

**Reconstructions**

Ancestral character states have been reconstructed using the computer program MacClade (Maddison and Maddison, 1992), which finds the most parsimonious



**Fig. 3.** A. Maximum parsimony cladogram based on combined DNA sequences of paralogs I and II for the nuclear gene for actin (Carlini, 1998; Carlini *et al.*, 2000); tree length 1581, consistency index 0.461, retention index 0.549. Branch lengths are proportional to changes in nucleotide sequence. B. Cladogram "pruned" for character-state reconstruction to retain nodal structure but reduce terminal taxa to those for which morphological data were assessed.

reconstructions. A characteristic of MacClade must be noted, however. In the case of polytomies, such as the decapod clade in the morphological tree, MacClade attempts to reconstruct character evolution as if the polytomy were resolved dichotomously by inventing an intercalated node between the polytomy and the basal dichotomous node outside of the polytomy. This convention is used when a character is polymorphic within a polytomy because the context of the basal node will vary with different resolutions of the phylogeny. However, for cases in which all taxa within a polytomy share a character state, we consider that state to be ancestral to the polytomy, rather than equivocal as

would be reconstructed by MacClade. In the current presentation, this convention does not apply to the molecular trees, which are fully resolved, but affects many reconstructions based on the morphological tree, especially for the ancestral decapod node.

Character states were considered to be unordered except for three characters for analysis on the morphological tree. Young and Vecchione (1996) treated characters number 8 (arms II), 35 (superior buccal lobe) and 36 (subfrontal lobe) as ordered to infer the morphological phylogeny used here. Because these characters were considered to be ordered for development of the tree, we have treated



them as ordered for character-state reconstruction based on the morphological tree. However, rather than impose these morphological assumptions on the independent molecular analyses, we considered all character states to be unordered for reconstructions based on gene trees.

To determine the degree of agreement among trees, we define a series of rules for the consensus column in Tables 1-3. (1) Strong agreement occurs when all three trees indicate the same character state at a node. (2) Moderate agreement is when two trees agree on a character state and the third reconstructs the character state as equivocal. (3) If two trees agree on a character state and the third reconstructs the character as an alternative state (as in majority-rules consensus) we consider this to be weak agreement. (4) When the reconstructed state is equivocal on two or three trees, this is listed as lack of consensus. (5) Disagreement occurs when two trees reconstruct a character as having different states and the third indicates that the state is equivocal.

## RESULTS

Reconstructions of the states of 51 characters on morphological, nuclear (actins I and II), and mitochondrial (COI) trees are summarized in Table 1 for the ancestral neocoleoid node, Table 2 for the ancestral octopodiform node, and Table 3 for the ancestral decapod node. Of the total of 153 characters-at-nodes, strong agreement (all three trees agree on a character state) was found at 97, or 63.4%. If moderate agreement is included, the number increases to 112 (73.2%). Overall, the amount of agreement among trees, including strong, moderate, and weak agreement, was high at the decapodiform (43 characters) and octopodiform nodes (41 characters) and much lower (30 characters) at the neocoleoid node. The maximum strong agreement (38 characters) and least disagreement (1 character) among trees was found at the ancestral octopodiform node. Although the ancestral decapod node had a higher number of characters with strong agreement among trees (34) than did the ancestral neocoleoid node (25), the neocoleoid node had only two characters for which the trees disagreed; there were five such characters at the decapod node.

For some characters, agreement among trees was consistently strong at all focus nodes. The inferred character state remained unchanged among nodes for some of these characters. Examples include character 5, fins, character 10, suckers, and character 14, arm trabeculae, all present at all nodes. Other characters for which strong agreement was consistent within nodes exhibited changes in inferred state among nodes. Examples of these include character 7, the buccal crown (present in ancestral decapods but absent in octopodiforms), and character 19, the outer capsule of the statocyst (absent in ancestral neo-

coleoids and decapods but present in octopodiforms).

Several characters are otherwise noteworthy. Reconstructions of character 47, the position of the intestine relative to the vena cava, resulted in disagreement among trees at all three focus nodes (Figs. 1-3). Similarly, head width reconstruction, character 49, resulted in disagreement at two nodes and only weak agreement at the third. Assessment of states for both of these characters was particularly difficult (Appendix 1; Young and Vecchione, 1996).

Character 1, the phragmocone, is an example of a problem that is perhaps more important. Parsimony reconstructed the phragmocone as either absent or equivocal at every focus node on every tree. As discussed below, the phragmocone almost certainly was present at least in ancestral neocoleoids and decapods and possibly was present also in ancestral Octopodiformes. Therefore, these reconstructions of this character are almost certainly either incorrect (absent in actins at these nodes) or uninformative (equivocal elsewhere). Similarly, the reconstruction of the digestive gland, character 44, as fused at all ancestral nodes can be questioned based on embryological evidence of a paired origin of this structure. Such problems raise questions of reliability of the other reconstructions. We therefore present in Appendix 3 a summary of hypothesized ancestral character states based on ontogenetic and paleontological evidence in addition to parsimony, as discussed in Appendix 1.

## DISCUSSION

Some neocoleoid morphological character states can be polarized by the extant outgroup, *Nautilus*, fossil cephalopods, or cephalopod embryology, but many cannot be polarized due to the lack of a living, closely-related outgroup for neocoleoids. Sufficient information exists, however, to make a first attempt at defining some of the basic character states (Appendix 1). The morphological hypothesis of relationships used here suffers from a lack of resolution within decapod and incirrate octopod clades. As a result, inferences are equivocal for character-state changes of many characters. The molecular analyses do not have this problem but suffer from unsubstantiated (*i. e.*, lack of bootstrap support) subclade relationships. Whereas the tree derived from the actin genes has the same major nodes as the morphological tree, the COI gene tree disagrees in the relationships within the Octopoda. Both molecular trees, however, are completely resolved within the Decapodiformes, although showing considerable disagreement between them, while the morphological tree depicts the Decapodiformes as an unresolved (*i. e.*, soft) polytomy. Although our analyses involve three quite different trees,

**Table 1.** Character states inferred using parsimony for the ancestral neocoleoid nodes of trees based on morphology, DNA sequence for combined actins I and II (of three paralogs), and DNA sequence for cytochrome oxidase subunit I. "Consensus" indicates agreement among trees with: --, indicating disagreement; -, indicating no consensus; a character state without an asterisk indicating weak agreement; \*, indicating moderate agreement; and \*\*, indicating strong agreement.

Character	Morphology	Actins	COI	consensus
1 Phragmocone	Equivocal	Absent	Equivocal	-
2 Proostracum	Present	Present	Equivocal	*
3 Median Field	Equivocal	Equivocal	Equivocal	-
4 Shell Number	1	1	1	**
5 Fins	Present	Present	Present	**
6 Fin Cartilage	Equivocal	Equivocal	Equivocal	-
7 Buccal Crown	Equivocal	Equivocal	Equivocal	-
8 Arms II	Unmodified	Equivocal	Equivocal	-
9 Arms IV	Unmodified	Equivocal	Equivocal	-
10 Suckers	Present	Present	Present	**
11 Sucker Stalks	Equivocal	Equivocal	Equivocal	-
12 Sucker symmetry	Equivocal	Equivocal	Equivocal	-
13 Sucker Rings	Equivocal	Equivocal	Equivocal	-
14 Arm Trabeculae	Present	Present	Present	**
15 Arm Protective Membrane	Equivocal	Present	Present	*
16 Arm III Sucker Series	Equivocal	Equivocal	Equivocal	-
17 Arm I Web	Present	Equivocal	Present	*
18 Arm V Web	Equivocal	Equivocal	Equivocal	-
19 Statocyst Outer Capsule	Absent	Absent	Absent	**
20 Nephridial Coelom	2	2	2	**
21 Visceropericardial Coelom	Extensive	Extensive	Extensive	**
22 Dorsal Mantle Cavity	Absent	Absent	Absent	**
23 Nidamental Glands	Equivocal	Present	Present	*
24 Crop	Present	Present	Present	**
25 Branchial Canal	Equivocal	Present	Present	*
26 Mantle Septum	Equivocal	Equivocal	Equivocal	-
27 Mantle Adductor	Present	Equivocal	Equivocal	-
28 Funnel Valve	Present	Present	Present	**
29 Nuchal Cartilage	Present	Present	Present	**
30 Cornea	Absent	Absent	Absent	**
31 Right Oviduct	Present	Present	Present	**
32 Oviducal Gland Symmetry	Equivocal	Equivocal	Equivocal	-
33 Oviducal Gland Position	Terminal	Terminal	Terminal	**
34 Photosensitive Vesicle	Equivocal	Equivocal	Equivocal	-
35 Superior Buccal Lobe	Broad Sep.	Broad Sep.	Broad Sep.	**
36 Subfrontal Lobes	Equivocal	Equivocal	Equivocal	-
37 Arm-Mantle Muscle	Absent	Absent	Absent	**
38 Horizontal Arm Septae	Absent	Absent	Absent	**
39 Arm IV (III) Hectocotylus	Absent	Absent	Absent	**
40 Arm V Hectocotylus	Absent	Equivocal	Equivocal	-
41 Spermatophores	Present	Present	Present	**
42 DGDA, Number	Equivocal	Equivocal	Equivocal	-
43 DGDA, Location	Equivocal	Equivocal	Equivocal	-
44 Digestive Gland	Fused	Fused	Fused	**
45 Gonad: Coelom Coverage	Mostly Cov.	Mostly Cov.	Mostly Cov.	**
46 Posterior Salivary Gland	Post.to	Post.to	Post.to	**
47 Intestine vs Vena Cava	Equivocal	Dorsal	Ventral	--
48 Gill Filaments	Both Free	Both Free	Both Free	**
49 Head Width	1.00-1.49	Equivocal	0.50-0.99	--
50 Long. Mantle Muscle	Present	Present	Present	**
51 Arm Orientation	Anterior	Anterior	Anterior	**

**Table 2.** Character state inferences as in Table 1, but for the ancestral Octopodiformes nodes.

Character	Morphology	Actins	COI	consensus
1 Phragmocone	Absent	Absent	Absent	**
2 Proostracum	Present	Present	Equivocal	*
3 Median Field	Equivocal	Equivocal	Equivocal	-
4 Shell Number	1	1	1	**
5 Fins	Present	Present	Present	**
6 Fin Cartilage	Base + Core	Base + Core	Base + Core	**
7 Buccal Crown	Absent	Absent	Absent	**
8 Arms II	Modified	Equivocal	Equivocal	-
9 Arms IV	Unmodified	Unmodified	Unmodified	**
10 Suckers	Present	Present	Present	**
11 Sucker Stalks	Equivocal	Equivocal	Equivocal	-
12 Sucker symmetry	Radial	Radial	Radial	**
13 Sucker Rings	Equivocal	Equivocal	Equivocal	-
14 Arm Trabeculae	Present	Present	Present	**
15 Arm Prot. Membrane	Equivocal	Present	Present	*
16 Arm III Sucker Series	1	1	1	**
17 Arm I Web	Present	Present	Present	**
18 Arm V Web	Present	Present	Present	**
19 Statocyst Outer Capsule	Present	Present	Present	**
20 Nephridial Coelom	2	2	2	**
21 Visceropericardial Coelom	Extensive	Extensive	Extensive	**
22 Dorsal Mantle Cavity	Absent	Absent	Absent	**
23 Nidamental Glands	Absent	Absent	Absent	**
24 Crop	Present	Present	Present	**
25 Branchial Canal	Present	Present	Present	**
26 Mantle Septum	Equivocal	Equivocal	Equivocal	-
27 Mantle Adductor	Present	Equivocal	Equivocal	-
28 Funnel Valve	Present	Present	Present	**
29 Nuchal Cartilage	Present	Present	Present	**
30 Cornea	Absent	Absent	Absent	**
31 Right Oviduct	Present	Present	Present	**
32 Oviducal Gland Symmetry	Radial	Radial	Radial	**
33 Oviducal Gland Position	Terminal	Terminal	Terminal	**
34 Photosensitive Vesicle	Equivocal	Equivocal	Equivocal	-
35 Superior Buccal Lobe	Adjacent	Equivocal	Equivocal	-
36 Subfrontal Lobes	Incipient	Equivocal	Equivocal	-
37 Arm-Mantle Muscle	Absent	Absent	Absent	**
38 Horizontal Arm Septae	Absent	Absent	Absent	**
39 Arm IV (III) Hectocotylus	Absent	Absent	Absent	**
40 Arm V Hectocotylus	Absent	Absent	Absent	**
41 Spermatophores	Present	Present	Present	**
42 DGDA, Number	Single	Single	Single	**
43 DGDA, Location	Not in coel	Not in coel	Not in coel	**
44 Digestive Gland	Fused	Fused	Fused	**
45 Gonad: Coel. Coverage	Mostly Cov.	Mostly Cov.	Mostly Cov.	**
46 Posterior Salivary Gland	Post.to	Post.to	Post.to	**
47 Intestine vs Vena Cava	Equivocal	Dorsal	Ventral	--
48 Gill Filaments	Both Free	Both Free	Both Free	**
49 Head Width	1.00-1.49	0.50-0.99	0.50-0.99	**
50 Long. Mantle Muscle	Present	Present	Present	**
51 Arm Orientation	Lateral	Lateral	Lateral	**

agreement among them in reconstructing specific character states provides some confidence in the reconstruction.

The Octopodiformes clade, which is most consistently resolved among the three trees, resulted in the most consistent reconstructions. The decapod clade, which was

totally unresolved in the morphological tree considered here and was inconsistently resolved between the two molecular trees, resulted in many disagreements in reconstructed character states. The deeper node for ancestral neocoleoids was most noteworthy because of the large number

**Table 3.** Character state inferences as in Table 1, but for the ancestral Decapodiformes nodes.

Character	Morphology	Actins	COI	consensus
1 Phragmocone	Equivocal	Absent	Equivocal	-
2 Proostracum	Present	Present	Equivocal	*
3 Median Field	Equivocal	Narrow	Equivocal	-
4 Shell Number	1	1	1	**
5 Fins	Present	Present	Present	**
6 Fin Cartilage	Base Only	Base Only	Base Only	**
7 Buccal Crown	Present	Present	Present	**
8 Arms II	Unmodified	Unmodified	Unmodified	**
9 Arms IV	Tentacles	Tentacles	Tentacles	**
10 Suckers	Present	Present	Present	**
11 Sucker Stalks	Base & Neck	Base & Neck	Base & Neck	**
12 Sucker symmetry	Bilateral	Bilateral	Bilateral	**
13 Sucker Rings	Horny	Horny	Horny	**
14 Arm Trabeculae	Present	Present	Present	**
15 Arm Protective Membrane	Equivocal	Present	Present	*
16 Arm III Sucker Series	Equivocal	Equivocal	Equivocal	-
17 Arm I Web	Present	Absent	Present	
18 Arm V Web	Absent	Absent	Absent	**
19 Statocyst Outer Capsule	Absent	Absent	Absent	**
20 Nephridial Coelom	1	1	1	**
21 Visceropericardial Coelom	Extensive	Extensive	Extensive	**
22 Dorsal Mantle Cavity	Absent	Absent	Absent	**
23 Nidamental Glands	Equivocal	Present	Present	*
24 Crop	Absent	Absent	Absent	**
25 Branchial Canal	Equivocal	Present	Absent	--
26 Mantle Septum	Equivocal	Continuous	Continuous	*
27 Mantle Adductor	Present	Absent	Equivocal	--
28 Funnel Valve	Present	Present	Present	**
29 Nuchal Cartilage	Present	Present	Present	**
30 Cornea	Absent	Absent	Equivocal	*
31 Right Oviduct	Present	Present	Equivocal	*
32 Oviducal Gland Symmetry	Bilateral	Bilateral	Bilateral	**
33 Oviducal Gland Position	Terminal	Terminal	Terminal	**
34 Photosensitive Vesicle	Equivocal	In Cart.	In Cart.	*
35 Superior Buccal Lobe	Br.Separate	Br.Separate	Br.Separate	**
36 Subfrontal Lobes	Equivocal	Absent	Absent	*
37 Arm-Mantle Muscle	Absent	Absent	Absent	**
38 Horizontal Arm Septae	Absent	Absent	Absent	**
39 Arm IV (III) Hectocotylus	Absent	Absent	Absent	**
40 Arm V Hectocotylus	Absent	Present	Equivocal	--
41 Spermatophores	Present	Present	Present	**
42 DGDA, Number	Paired	Paired	Paired	**
43 DGDA, Location	Nephrocoel	Nephrocoel	Nephrocoel	**
44 Digestive Gland	Fused	Fused	Fused	**
45 Gonad: Coelomic Coverage	Covered	Covered	Covered	**
46 Posterior Salivary Gland	Post.to	Post. to	Post. to	**
47 Intestine vs Vena Cava	Equivocal	Dorsal	Ventral	--
48 Gill Filaments	Both Free	Both Free	Both Free	**
49 Head Width	1.00-1.49	0.00-0.49	Equivocal	--
50 Long. Mantle Muscle	Present	Present	Present	**
51 Arm Orientation	Anterior	Anterior	Anterior	**

of characters (19) for which there was no consensus among reconstructions, a result of the many characters for which reconstructions on individual trees were equivocal.

Reconstruction of ancestral character states using parsimony is a three-step process, including down-tree opti-

mization, up-tree optimization, and final optimization reconciling the previous two steps (see Box 1 in Cunningham *et al.*, 1998 for a more complete explanation). As a result, changes in resolution of a subclade can have repercussions at nodes much deeper in a cladogram. For example, if the



traditional, but controversial, suborder Sepioidea (= Sepiidae + Sepiadariidae + Sepiolidae + Idiosepiidae + Spirulidae) is considered to be a subclade within the Decapodiformes on the morphological cladogram used here, change in character states inferred at the ancestral decapod and neocoleoid nodes may (*e. g.*, character 3, median field of the proostracum, changes from absent to equivocal) or may not (*e. g.*, character 2, presence of the proostracum, is unchanged) occur, depending on the distribution of character states at the terminal branches. Parsimony-based reconstruction is also sensitive to assumptions about rates of evolution and probabilities of gain and losses (Cunningham *et al.*, 1998), questions about which very little information exists for neocoleoid cephalopods.

It is encouraging that some of these reconstructions are consistent with other sources of information. For instance, the reconstructed presence of fins, suckers, and arm trabeculae agree with inferences from the fossil record (Bandel and Leich, 1986). However, inability of parsimony to reconstruct the phragmocone as present based on the current phylogenetic hypotheses is troubling. A phragmocone is present in cuttlefish and *Spirula*, as well as in the extant outgroup, *Nautilus*. Additionally, phragmocones are known from fossil coleoids, including the belemnoid outgroup and early spirulids. It seems unlikely that such a complex structure evolved independently in all of these groups. Therefore, ancestral decapods and neocoleoids almost certainly possessed a phragmocone. *Vampyroteuthis* also has a structure of unknown function that could be a remnant of the siphuncle from a phragmocone (Young and Vecchione, 1996), indicating the possibility that ancestral octopodiforms may also have retained a phragmocone. The reconstructed state of this character as either absent or equivocal, together with the reconstructed state of the digestive gland, which contradicts embryological evidence (Appendix 1), greatly reduced our confidence in parsimonious reconstructions of ancestral character states based on our current knowledge of cephalopod phylogeny.

Character reconstruction requires known phylogenetic relationships. The analyses presented here, however, involve three trees depicting somewhat different phylogenetic relationships. The reconstruction of morphology in Appendix 3 is based on the assumption that the following relationships are correct: (1) The Belemnoidea and Neocoleoidea are sister groups (Young *et al.*, 1998). The apomorphic character states of the Neocoleoidea are: A. presence of suckers; B. absence of a nacreous layer in the shell; C. presence of fins. This foundation, while presently convincing, requires confirmation. (2) The Octopodiformes and Decapodiformes are monophyletic sister groups. Monophyly of the decapods was supported by a morphological cladistic study (Young and Vecchione, 1996) and molecular studies (Bonnaud *et al.*, 1997; Carlini, 1998;

Carlini and Graves, 1999). Morphological support for monophyly was weak. The sole unambiguous morphological character found to unite the decapods was the modification of the fourth pair of arms into tentacles (belemnoids such as *Jeletzkyia* and *Belemnotheutis* had 10 equal arms, presumably the primitive condition) although they share a variety of characters that could not be polarized. The monophyly of the Octopodiformes (Octopoda + Vampyromorpha; see Young *et al.*, 1998 for discussion of the proper name of this clade) was supported morphologically by: A. the shared outer capsule of the statocyst; B. modification of the second pair of arms; C. the position of the superior buccal lobe of the brain. Extant octopods have lost one pair of arms but the lost pair, apparently, is not the tentacles (arms IV), but rather arms II, which became retractile filaments in the Vampyromorpha; this problem is discussed in more detail by Young and Vecchione (1996; 1999) and Vecchione *et al.* (1999). (3) The Vampyromorpha and Octopoda are sister groups within the Octopodiformes. This genealogy has now been confirmed by separate cladistic studies of morphological and molecular data (Young and Vecchione, 1996; Bonnaud *et al.*, 1997; Carlini and Graves, 1999). (4) The Cirrata and Incirrata are sister groups within the Octopoda. This relationship has been supported by morphology (Young and Vecchione, 1996; Voight, 1997). A sister-group relationship between the cirrates and incirrates is not supported by COI data (Carlini and Graves, 1999). This relationship was not adequately tested by the actin data in Carlini (1998) because few cirrates were included in the analysis due to difficulties in cloning cirrate actin DNA. However, the few cirrate taxa sampled for actin genes suggest a sister-group relationship between cirrates and incirrates. Furthermore, monophyly of the Octopoda is supported by both actin and COI.

Although reconstruction of ancestral character states is, of necessity, speculative (Frumhoff and Reeve, 1994), Shultz *et al.* (1996) concluded that such inferences can be remarkably robust. We have only begun the process of reconstruction here. Our understanding of coleoid evolution needs: (1) addition of characters to the list presented here, (2) resolution of the phylogenetic relationships among the decapods, the cirrates, and the incirrates and (3) greater knowledge of the developmental history of these characters in the embryos of all families considered. The latter will greatly increase our ability to define, assess, and polarize characters and clarify reconstructions that are presently ambiguous.

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## APPENDIX 1. Comments on individual characters.

1. Phragmocone. States: 0- Present; 1- Absent. Young and Vecchione (1996) considered only the character "siphuncle." We expand the definition here to "phragmocone," which includes a siphuncle. The presence of a calcareous phragmocone in belemnoid fossils and *Nautilus* indicates that this structure was present in the ancestral coleoid. Among Neocoleoidea only sepiids and *Spirula* have a phragmocone. Since the highly complex phragmocone is unlikely to have arisen anew in neocoleoid evolution, we consider this an irreversible character. Pickford (1940) and Young and Vecchione (1996) have described what could be a remnant of a siphuncle in *Vampyroteuthis*. If correct, this supports the presence of a phragmocone in the early octopodiform. This possibility, however, is uncertain so the reconstruction of the early octopodiform, for now, must be "absent."

2. Proostracum. States: 0- Present; 1- Absent; 2- Entire. The coleoid proostracum (*i. e.*, the dorsal remnant of the living chamber of the pre-coleoid) is considered to be homologous with the gladius, except conus, in decapods and *Vampyroteuthis*. A proostracum does not exist in sepiids or *Spirula*. Some sepiolids (*e. g.*, *Rossia*) have a small anterior gladius that appears to represent the anterior end of the proostracum based on its embryonic development (Naef, 1921-1923). Other sepiolids and sepiadariids that lack a gladius obviously lack a proostracum. In octopods the internal shell, the apparent homologue of the gladius, is transversely elongate; it is single in cirrates and divided, when present, in incirrates. The structure of the octopod shell, however, cannot be related to the subdivisions seen in the gladius and the state of the proostracum in octopods coded as a "?". A proostracum is present in belemnoids. The complete living chamber of *Nautilus* is given the state "Entire." A narrow proostracum is present in the fossil sepioid *Groenlandibelus* and, therefore, absence in *Sepia* and *Spirula* can be interpreted as a loss.

3. Median field width of proostracum. States: 0- Broad; 1- Narrow; 2- Unrecognizable; 3- Absent; 4- Entire (*Nautilus*). In many extant coleoids, the gladius has a three-part structure: a medial field (= rhachis), lateral fields (= wings) and conus fields (these often form part of the terminal primary conus but may extend well anterior of the conus proper). A primary conus can be present or absent. The primary conus and conus fields are presumably remnants of the phragmocone while the rest of the gladius represents the proostracum. The anterior portion of the gladius of extant coleoids consists solely of the median field, which can be narrow (teuthoids and some sepioids) or broad in *Vampyroteuthis*. Belemnoids also have a broad proostracum, but its relationship to the median field is also uncertain so this has been coded as "?".

4. Shell number. States: 0- None; 1- One; 2- Two. The shell can be single (*i. e.*, teuthoid gladius) or double (incirrate shell) or absent (some sepioids, incirrates). The double break in the mantle musculature in the position of stylets in the two incirrate families that lack stylets clearly indicates that the double state is ancestral to these families. The single condition as in cirrates clearly is the ancestral state as the double state in incirrates is derived from a single shell sac during embryology (Naef, 1921-1923). This interpretation contrasts with that of Voight (1997) that the double shell was ancestral to the Octopoda. We therefore consider this an ordered character and, as such, reconstruct the single condition as the ancestral incirrate condition.

5. Fins. States: 0- Present; 1- Absent. Fins are unknown in belemnoids (Young *et al.*, 1988), but are found throughout the decapods, as well as in *Vampyroteuthis* and the cirrate octopods, but are absent in incirrates. *Vampyroteuthis* has two pairs of fins. In the youngest specimens a juvenile fin is present. As the animal grows, a second pair of fins develops anteriorly, which persist into the adult stage; the juvenile fins are resorbed. Young and Vecchione (1996) presented evidence that the juvenile fin is the homologue of other cephalopod fins. MacClade reconstructs the ancestral incirrate condition as equivocal. However, fin folds

are present in incirrate embryology (Naef, 1921-1923) suggesting that early incirrates had fins. In addition, fins are present in the fossil *Palaeoctopus newboldi*, which has been interpreted as an incirrate (Engeser and Bandel, 1988; Young *et al.*, 1998).

6. Fin cartilage. States: 0- Base only; 1- Base and core; 2- Absent. Both *Vampyroteuthis* (juvenile fin) and the cirrates have an extensive core of flexible cartilage extending through half or more of the fin (Young and Vecchione, 1996). This is absent from decapods, which have a cartilage at the base of each fin that doesn't penetrate the fin core. Incirrates lack fins and are coded as "?". Because the Decapodiformes are considered an unresolved bush in the morphological consensus tree, MacClade cannot determine whether the uniform decapod condition occurred at the node of the bush or earlier.

7. Buccal crown. States: 0- Oral arms; 1- Present; 2- Absent. The buccal crown is present in decapods and absent in octopodiforms. It is thought to be homologous with the oral arms of *Nautilus* (Young and Vecchione, 1996). We therefore consider the states to be ordered: oral arms - buccal crown - absent. With this constraint, the ancestral neocoleoid condition is reconstructed as "present." The state in belemnoids is unknown. However, since the arm crown of belemnoids is known and oral arms are not present the options are either state 1 or 2.

8. Arms II. States: 0- Unmodified; 1- Modified; 2- Absent. Arms II are modified in *Vampyroteuthis* and lost in octopods. The likelihood that these assumptions are correct is discussed in Young and Vecchione (1996). On the assumption that modification preceded loss, we order the states, which then reconstructs the ancestral octopodiform as "modified."

9. Arms IV. States: 0- Unmodified; 1- Modified (tentacles). Arms IV are modified as tentacles in decapods but are unmodified in octopodiforms. In several decapod species adults have only eight arms due to the loss of the tentacles during ontogeny. The eight-armed condition in these decapods is clearly secondary as tentacles are present in paralarvae.

10. Suckers. States: 0- Absent; 1- Present. *Nautilus* lacks suckers as, apparently, did the belemnoids. Donovan and Crane (1992) reported possible suckers in *Belemnotheruthis*, but these are more likely the muscular bases of hooks (Young *et al.*, 1998). Because all living coleoids have suckers, we consider the presence of suckers to be a neocoleoid synapomorphy as suggested by Engeser and Bandel (1988) and as reconstructed by MacClade.

11. Sucker stalks. States: 0- Base and neck; 1- Base and plug; 2- Cylinder. The suckers of decapods have stalks that are cone-like and terminate in a constricted, narrow neck. Octopods have broad, cylindrical sucker stalks. *Vampyroteuthis* has stalks that are unique (state 1), but in some ways intermediate between the decapod and octopod conditions. Polarity among these character states is presently undetermined.

12. Sucker symmetry. States: 0- Radial; 1- Bilateral. Decapods are characterized by having bilaterally symmetrical suckers. Octopods and *Vampyroteuthis* have suckers that are radially symmetrical, and reconstruction of the ancestral octopodiform is therefore with radial suckers. Polarity among these character states is presently undetermined.

13. Sucker rings. States: 0- Cuticular; 1- Absent; 2- Horny. Octopods have cuticular sucker rings; decapods have horny sucker rings often modified into hooks, and *Vampyroteuthis* lacks sucker rings. Polarity among these character states is presently undetermined.

14. Arm trabeculae. States: 0- Present; 1- Absent. Trabeculae (including their apparent homologues, cirri) are present in many decapods, *Vampyroteuthis* and the cirrate octopods. Within decapods, however, trabeculae are often reduced or absent. Belemnoids and *Nautilus* were coded as "?"; belemnoids because of uncertainty and *Nautilus* because the arms are so different that the character is not applicable. Haas (1989) has proposed that belemnoid arm hooks could be homologous with



neocoleoid trabeculae.

15. Arm protective membranes. States: 0- Present; 1- Absent. Although protective membranes are present between distal trabeculae on the arms of *Vampyroteuthis*, they are completely absent from octopods and from one decapod family (Sepiolidae); all other decapods examined possess protective membranes although they are often reduced. We consider that the absence from the sepiolids is likely due to secondary loss (as reconstructed in the molecular data sets). Under this constraint, the ancestral decapod, octopodiform and neocoleoid are reconstructed as "present."

16. Armature series on arms III. States: 0- One; 1- Two; 2- Four; 3- > Four. In Octopodiformes the suckers are either one or two series, or occasionally a combination of these states. However, many incirrates with two series of arm suckers have a single series as hatchlings. This supports a reconstruction of a single series in the ancestral octopodiform. Recent decapods have their armature in two, four or sometimes more than four series. In decapods, as in octopods with two series, the suckers are staggered, suggesting a sequence in which single series become double series and double series become quadruple series (as noted by Naef, 1921-1923). Unfortunately there are no concrete data to support this hypothesis and the condition in the ancestral decapod must be reconstructed as equivocal. In *Vampyroteuthis* and the cirrates, there are two trabeculae/sucker on the sucker-bearing portion of the arms. In decapods where two sucker series exist, there is one trabecula/sucker; where four sucker series are present, there is one trabecula/two suckers. Trabeculae, therefore, appear to be progressively lost as the number of sucker series increases. That is, the trabecula is lost from the side of the sucker that no longer is adjacent to the margin of the arm. This is a scenario that would be expected as one series becomes multiple series.

17. Arm webs, dorsal. States: 0- Absent; 1- Present. Well-developed webs between the dorsal pair of arms are present in most octopodiforms and some decapods. We consider the dorsal sector to be representative of web development between the dorsal six arms.

18. Arm webs, ventral. States: 0- Absent; 1- Present. A web between the ventral arms is present in all major octopodiform lineages, but is uniformly absent in decapods.

19. Outer capsule of statocyst. States: 0- Absent; 1- Present. An outer capsule is present in the statocyst of *Vampyroteuthis* and all octopods. It is absent in decapods and *Nautilus*.

20. Nephridial coelom. States: 0- Two; 1- One. Octopodiforms and *Nautilus* have two nephridial coeloms (one pair), whereas one (fusion) coelom is uniform in decapods. This character was not used by Young and Vecchione (1996) to support decapod monophyly due to difficulties in determining if fusion occurred more than once.

21. Visceropericardial coelom. States: 0- Extensive; 1- Reduced. An extensive visceropericardial coelom is found in *Nautilus*, the decapods and *Vampyroteuthis*. Reduction in octopods is a synapomorphy in this clade.

22. Dorsal mantle cavity. States: 0- Absent; 1- Present. The dorsal mantle cavity as defined by Young and Vecchione (1996) is an autapomorphy in the Octopoda. This structure bears considerable resemblance to the large nuchal cavity of *Spirula* and we suspect that both were derived in the same manner. The nuchal cavity is a space where the dorsal mantle articulates with the head. In most decapods the gladius (= proostracum) extends to the anterior tip of the mantle where a cartilaginous reinforcement of the shell sac articulates with the nuchal cartilage. *Spirula* lacks a proostracum and the Recent octopod shell doesn't reach the anterior mantle margin, yet a proostracum was probably present in the ancestors of both groups (see character no. 2). Perhaps the proostracum was progressively reduced over evolutionary time, and as the proostracum receded posteriorly, the nuchal cavity increased accordingly. The later anterior fusion of the head and mantle margin in the Octopoda formed the dorsal mantle cavity. This scenario is further supported by the reduced gladius found in the Idiosepiidae. Here the gladius is absent both anteriorly and

posteriorly. Anteriorly, an expanded nuchal cavity is also present. The convergent condition in *Spirula* and *Idiosepius* occurred without the dorsal mantle and head fusing. For species in which the head-mantle fusion has occurred without a posterior regression of the gladius (e.g., *Sepioida*, *Vampyroteuthis*), the nuchal cavity has been obliterated.

23. Nidamental glands. States: 0- Absent; 1- Present. These glands produce some of the external coatings on eggs as they are spawned. The glands are found in *Nautilus* and nearly all decapods except the Enoploteuthidae. Because of their absence in the latter family, reconstructions of the ancestral neocoleoid and decapod based on morphology are equivocal. However, we consider that the absence in the Enoploteuthidae, which spawn individual eggs strung together in a single gelatinous strand rather than gelatinous or encapsulated egg masses, is probably a secondary loss (as predicted by the molecular trees). It is unlikely that nidamental glands evolved twice (i. e., decapods and *Nautilus*). With this constraint we reconstruct the ancestral decapod as having nidamental glands.

24. Crop. States: 0- Present; 1- Absent. A crop, defined here as a swelling or diverticulum of the esophagus, is present in *Nautilus*, *Vampyroteuthis* and most octopods. The loss of the crop, therefore, is an apomorphy for the Decapodiformes.

25. Branchial canal. States: 0- Absent; 1- Present; 2- Secondary loss. A branchial canal is present in teuthoids, *Vampyroteuthis* and in the incirrate octopods, but absent from *Nautilus*, some decapods (sepioids) and the cirrates. The cirrates, however, have highly modified gills, which likely resulted in the loss of the canal independent of the loss in some decapods; the cirrate condition, therefore, is coded as a different state. Because the condition in the Decapodiformes is polymorphic, the ancestral decapod state is equivocal.

26. Median mantle septum. States: 0- Absent; 1- Present and continuous; 2- Present but open posteriorly; 3- Present as a blood vessel only. The visceral mass of *Spirula* is highly distorted by the presence of a coiled phragmocone and the mantle septum is absent except for the presence of the median mantle artery. Because this artery normally passes along the anterior margin of the septum, we consider the artery to represent the mantle septum in *Spirula*. This makes the presence of the septum uniform within the decapods as it is in the octopods. It is absent in *Vampyroteuthis*. The septum is open in all octopods except the cirrate *Grimptoteuthis glacialis*.

27. Mantle adductor. States: 0- Absent; 1- Present. The mantle adductor is uniformly present in the octopods and in the sepiolid decapods. The sepiolids have a strong effect on the reconstructions because decapod relationships are unresolved. We consider that the mantle adductor in octopods and sepiolids is a result of convergence (as suggested by the molecular actin tree). Muscles are typically associated with the mantle septum and hypertrophy of these muscles into a mantle adductor in separate lineages could easily occur. Under this constraint, the ancestral decapod condition is reconstructed as "present."

28. Funnel valve. States: 0- Present; 1- Absent. The funnel valve is present in *Nautilus*, *Vampyroteuthis* and most decapods. Although it is absent among decapods in some cranchiids and *Planctoteuthis*, the presence of the funnel valve in some members of these families suggests that this is a secondary loss.

29. Nuchal cartilage. States: 0- Present; 1- Absent. Although *Nautilus* doesn't offer any information on this structure, the nuchal cartilage is present in *Vampyroteuthis* and nearly all decapods (it is absent in some sepiolids and sepiadariids; its presence in some members of these families indicates that these are secondary losses).

30. Cornea. States: 0- Absent; 1- One part; 2- Two part. A cornea is absent in the cirrate octopods and *Vampyroteuthis* as well as many decapods. A two-part cornea is present in the incirrates. A one-part cornea is present in some decapods. These two types of corneas are considered to be independent derivatives of the eyelid. We did not find any corneas in the cirrates examined. However, *Opisthoteuthis* possesses inner, muscular,



pigmented eyelids in the form of convex, overlapping horizontal membranes. These have the same form as the clear, fixed corneas of the incirrates and can be interpreted either as the forerunner or remnant of a cornea.

31. Right oviduct. States: 0- Absent; 1- Present. The right oviduct is present in many decapods (it is absent in loliginids and sepioids), *Vampyroteuthis*, incirrate octopods and *Nautilus*. This means that the oviducts were paired since the left oviduct is present in all neocoleoids. The absence of the right oviduct in cirrate octopods and some decapods represents convergence.

32. Oviducal gland symmetry. States: 0- Radial; 1- Bilateral; 2- Asymmetrical. The oviducal glands are radially symmetrical in the octopodiform lineage and bilaterally symmetrical in the decapod lineage but asymmetrical in *Nautilus*. The character therefore cannot be polarized.

33. Position of oviducal gland. States: 0- Terminal; 1- Subterminal. The oviducal gland is located in the terminal position in decapods, *Vampyroteuthis* and *Nautilus*, but is subterminal in the Octopoda.

34. Photosensitive vesicles. States: 0- Within cephalic cartilage; 1- Above funnel; 2- On stellate ganglia. In some decapods the photosensitive vesicles lie on the optic stalks of the brain, and in others they have moved off the stalks but lie mostly within the confines of the cephalic cartilage with nerves running to the optic stalks. The photosensitive vesicles lie on the stellate ganglia in octopods and their nerves pass through this ganglion and into the pallial nerve (J. Z. Young, 1977), which leads to the brain. In *Vampyroteuthis*, they lie just dorsal to the funnel and their nerves follow the posterior funnel nerve toward the brain. Thus, the three major lineages have vesicles in different localities. We suspect that nerves from photosensitive vesicles of all cephalopods enter the brain in the region of the optic stalk and that the vesicles originally evolved at this location. If so, the decapod state would be the plesiomorphic state for the Neocoleoidea. Unfortunately, this cannot be confirmed at present and this reconstruction is equivocal. The reconstruction further assumes that the unknown state in *Thysanoteuthis* will conform to that of other decapods.

35. Superior buccal lobes. States: 0- Broadly separated; 1- Adjacent; 2- Fused. The superior buccal lobes are far removed from the brain in *Nautilus* and decapods but are adjacent to the brain in *Vampyroteuthis* and are fused with the brain in octopods, with the greatest compaction occurring in the cirrate octopods. The situation in *Vampyroteuthis* is actually somewhat more intimate than "adjacent"; the lateral edges of the superior buccal lobe and posterior buccal lobes lie within the same connective tissue covering. The state of this character strongly reflects the distance between the brain and the buccal mass. Young and Vecchione (1996) considered this to be an ordered character: separate - adjacent - fused.

36. Inferior frontal system of the brain. States: 0- Absent; 1- Incipient; 2- Present. The inferior frontal system of incirrates deals with the use of chemotactile information from the arms (J. Z. Young, 1971). This system is composed of the posterior buccal, lateral inferior frontals, subfrontals and the median inferior frontal lobe (J. Z. Young, 1971). The system develops embryologically from the posterior buccal lobes (J. Z. Young, 1965) and is best developed in incirrate octopods, but is present in cirrates as well. In decapods only the posterior buccal lobes are present. In *Vampyroteuthis*, complexities of the posterior buccal lobes and their connections have been interpreted as an incipient inferior frontal system (J. Z. Young, 1977). We consider this to be an ordered character with the vampyromorph condition intermediate, as did Young and Vecchione (1996). The reconstruction further assumes that the unknown state in *Thysanoteuthis* will conform to that of other decapods.

37. Arm-mantle muscle. States: 0- Present; 1- Absent. Special muscle bundles run between the bases of the dorsal arms and the dorsal, anterior end of the mantle in the octopods. This feature is a synapomorphy of the octopods and defines the dorsal head-mantle fusion peculiar to

them. These muscles are not present in the head-mantle fusions of *Vampyroteuthis* or some decapods.

38. Horizontal arm septa. States: 0- Absent; 1- Present. Peculiar orally concave horizontal septa extend along the arms of all cirrate octopods and are found nowhere else. The arms of the incirrate bolitaenids have a somewhat similar arrangement but with different septal attachments; this condition was considered as a separate character state by Young and Vecchione (1996). Concave horizontal septa, therefore, is an apomorphy for the Cirrata.

39. Arm IV hectocotylization. States: 0- Absent; 1- Present. Because of the loss of arm pair II in octopods (see character 8), arms IV are generally considered by students of neocoleoids to be the "third" pair of arms. Modification of one of the "third" arms (actually arms IV) for the transmission of spermatophores is a synapomorphy in the incirrate octopods.

40. Arm V hectocotylization. States: 0- Absent; 1- Present. Modification of one of the ventral arms (arms V are often referred to as arms IV, not counting the tentacles in decapods as an arm pair) for the transmission of spermatophores occurs among many decapods, but not all, and is absent in other lineages.

41. Spermatophores. States: 0- Present; 1- Sperm packets; 2- Encapsulated coil. Typical spermatophores with an ejaculatory apparatus are found throughout the coleoids with the exception of the cirrate octopods. The presence of special sperm packets in cirrates, apparently a secondary simplification, is an apomorphy in this group.

42. Digestive-gland-duct appendages (DGDA), number. States: 0- Single; 1- Paired. In nearly all decapods the DGDA are spread along the ducts between the digestive gland and the caecum. In *Vampyroteuthis* and the octopods they are fused and compacted against the digestive gland. In a few genera of decapods (e. g., *Batoteuthis*, various cranchiids) compaction exists but not fusion. Because the appendages are lacking in *Nautilus*, polarity is uncertain.

43. DGDA, location. States: 0- In nephridial coelom; 1- Not in nephridial coelom. The DGDA in the decapods are located within (actually surrounded by) the dorsal sac of the nephridial coelom but they are separate from this coelom in the octopodiform lineage. The states cannot be polarized.

44. Digestive gland. States: 0- Many; 1- Paired; 2- Fused. Digestive glands are paired only in *Sepia*, *Spirula* and *Sepiadarium*. *Nautilus* has numerous digestive glands. Parsimony indicates the single state to be ancestral. However, embryology clearly indicates the paired origin of this structure in some species having a single organ (e.g., *Loligo*, *Octopus*). We therefore consider that paired glands are the ancestral neocoleoid state in spite of their reconstruction as fused. If this is correct, secondary fusion to produce a single digestive gland has occurred in more than one lineage.

45. Gonad: coelomic covering. States: 0- Mostly covered; 1- Less than 50% covered. In most neocoleoids the gonad lies suspended in the visceropericardial coelom (virtually 100% covered) although lined by the coelomic epithelium. In incirrates much less, but in excess of 50%, is covered. A synapomorphic condition exists in the cirrates in which less than 50% of the gonad lies within the coelom.

46. Posterior salivary glands. States: 0- Absent; 1- Posterior to cephalic cartilage; 2- On or in buccal mass. The posterior salivary glands are usually found posterior to the brain and the cephalic cartilage. Only in the cirrate octopods are they found anterior to the brain and on or in the buccal mass. Therefore, this latter state is synapomorphic in cirrates.

47. Intestine: position relative to vena cava. States: 0- Ventral; 1- Dorsal/anterior. The intestine either runs dorsal/anterior to the vena cava (*Vampyroteuthis*, sepioids, loliginids) or ventral to it (oegopsids and octopods). This character exhibits homoplasy (Young and Vecchione, 1996) and outside the Octopoda ancestral states are equivocal.

48. Gill filaments. States: 0- Both free; 1- Outer attached; 2-

Both attached. The tips of the gill filaments are free in some taxa (*Nautilus*, many decapods, *Vampyroteuthis*, some cirrates). Alternatively, one (*Onychoteuthidae*, *Ocythoidae*) or both (many incirrates, *Opisthoteuthidae*) filaments may be attached to the gill base.

49. Head width proportional to eye diameter. States: 0- 0-0.49; 1- 0.5-0.99; 2- 1.0-1.49; 3- 1.5-1.99; 4- 2.0-2.49; 5- 2.5-2.99; 6-3.0-3.49; 6- 3.5-3.99; 7- 4.0-4.49; 8- 4.5-4.99. Young and Vecchione (1996) attempted to quantify the head width by using the eye diameter as a size standard against which to measure head width. They compared the eye diameter to the head width measured between the extremities of the lenses and expressed it as a ratio. The method was only partially satisfactory because animals with dorsally tilted eyes added a complication and, in some, the eye size is simply a poor size standard for judging head width. Because of these problems, results of reconstruction must be taken cautiously. A general pattern, nevertheless, exists with many of the oegopsids (and *Spirula*) having narrow heads, most sepioids, loliginids and *Vampyroteuthis* having intermediate head widths and octopods having broad heads. The head of *Vampyroteuthis* is actually rather broad but since the eyes are especially large in this species our measure doesn't reflect head size very well. Head width, in addition, seems to be a good, but not absolute, indicator of body width.

50. Longitudinal mantle muscles. States: 0- Present; 1- Absent. Mantles of many decapods are composed mostly of circular and radial muscles but thin, discontinuous layers of longitudinal muscles are also

present on the outer surface of the mantle especially near the anterior and posterior ends of the mantle. All groups examined, with the exception of a few families of decapods, had longitudinal muscles.

51. Arm orientation. States: 0- Lateral; 1- Anterior. In the relaxed position, the arms of some cephalopods extend laterally away from the head while in others they extend anteriorly. The arms of all cephalopods, however, are very muscular and capable of moving through a wide range in orientation. We have searched for anatomical correlates (*e. g.*, how the arms relate to the buccal mass) of the two basic orientations in order to quantify the character states. We have, unfortunately, been unsuccessful; as a result this character has not been adequately surveyed. Nevertheless, there seems to be little question that a basic difference in arm orientation exists between the octopodiform lineage and the decapods. When the arms of the former (typically oriented laterally) bend forward, their base near the buccal mass generally extends first laterally then anteriorly as the arm curves forward. In contrast, one usually finds that, in decapods, arms are typically directed forward and when they are directed laterally the orientation at the base generally extends first anteriorly then laterally as the arm curves aborally. The lateral orientation is most obvious in *Vampyroteuthis* and the cirrate octopods. The difference is less obvious in some of the muscular pelagic octopods such as *Ocythoe*. *Nautilus* tentacles and the preserved arms of some belemnoids (*e. g.*, *Belemnotheutis*) show anteriorly oriented arms.

**APPENDIX 2.** Matrix of morphological character states used for reconstructions. Explanation of numerical designations for character states is presented in Appendix 1. Most of the material examined is listed in Young and Vecchione (1996).

Character	0		1					2					3					4					5				
	1	2	3	4	56789012345	6	7	89	0	123	4	5	6	78	9	012345678901234567	8	9	01								
Bathyteuthidae	1	0	1	1	00101101210	2	1	00	1	001	1	1	1	00	0	011000010000102011	0	0	01								
Enoploteuthidae	1	0	1	1	00101101200	1	0	00	1	000	1	1	1	00	0	011000010010102011	0	0	01								
Gonatidae	1	0	1	1	00101101200	2	0	00	1	001	1	1	1	00	0	011000010011?02011	0	0	11								
Loliginidae	1	0	1	1	00101101200	1	0	00	1	001	1	1	1	00	0	101000010010102010	0	(12)	01								
Ommastrephidae	1	0	1	1	00101101200	1	0	00	1	001	1	1	1	00	0	011000010010102011	(01)	0	11								
Onychoteuthidae	1	0	1	1	00101101210	1	0	00	1	001	1	1	1	00	0	011000010000102011	1	0	01								
Sepiidae	0	1	3	1	00101101200	2	1	00	1	001	1	0	1	00	0	101000010010101010	0	2	01								
Sepiolidae	1	(01)	(13)	(01)	00101101211	(12)	(01)	00	1	001	1	0	1	10	(01)	101000010000102010	0	1	01								
Spirulidae	0	1	3	1	00101101210	2	1	00	1	001	1	0	3	00	0	001000010010101010	0	0	01								
Thysanoteuthidae	1	0	1	1	00101101200	1	0	00	1	001	1	1	1	00	0	0110??10010102011	0	0	11								
Bolitaenidae	1	1	?	0	1?220120011	0	1	11	0	110	0	1	2	11	1	21012220010?012011	2	3	00								
Octopodidae	1	?	?	2	1?220120011	(01)	1	11	(01)	110	0	1	2	11	1	210122200100012011	2	5	00								
Ocythoidae	1	1	?	0	1?220120011	1	0	01	0	110	0	1	2	11	1	210122200100012011	1	4	01								
Cirroteuthidae	1	?	?	1	01220120001	0	1	11	0	110	(01)	2	2	11	1	000122201001012121	0	4	00								
Opisthoteuthidae	1	?	?	1	01220120001	0	1	11	0	110	0	2	(12)	11	1	0001?2201001012121	2	?	?0								
Vampyroteuthidae	1	0	0	1	01210110100	0	1	11	0	000	0	1	0	?0	0	010011110000012010	0	2	00								
Nautilidae	0	2	4	1	1?0?0?0?0?0?	?	?	?0	0	001	0	0	?	?0	?	0120?0?10?2?000?	0	9	01								
belemnoid	0	0	?	1	1?2000?0?0?0?	?	?	?0	?	???	?	?	?	?0	?	????????????????	?	?	?1								

